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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/566,886	02/01/2006	David M. Neville	14028.0295U2	9182

36339 7590 11/25/2009

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EXAMINER	
MARVICH, MARIA	
ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
11/25/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/566,886	NEVILLE ET AL.	
	Examiner	Art Unit	
	MARIA B. MARVICH	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *7/23/09*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-40* is/are pending in the application.

4a) Of the above claim(s) *27-38* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26,39 and 40* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01 February 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This office action is in response to an amendment filed 7/23/09. Claims 1-40 are pending in this office action. Claims 27-38 are withdrawn and therefore claims 1-26, 39 and 40 are under examination.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/23/09 has been entered.

The amendment has been persuasive in overcoming the rejection under 35 USC, 112 first paragraph for lack of written description.

Petition

Applicants' petition for revival of an application for patent abandoned unintentionally under 37 CRR 1.137(b) filed 7/23/09 has been granted.

Claim Objections

Claim 1, 12-14, 25 and 26 are objected to because of the following informalities (**These are new objections)** for clarification and to provide proper antecedent basis, the following amendment is recommended in claims 1, 25 and 26,

--.A method of expressing an immunotoxin in a *Pichia pastoris* that express the immunotoxin, the method comprising

a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract, --.

Secondly, in claim 1, step b) the claim should recite -- on the *Pichia pastoris* in the growth medium wherein the methanol induction is performed at a temperature of 16.5°C or below. -- This amendment is recommended to first establish that the induction is on the growing cell in growth medium as claims recited using "comprising" have not set order. Secondly, the word "of" makes the sentence grammatically correct.

In claim 12, reference to --the *Pichia pastoris*-- in line 1 is recommended. Furthermore, it is not clear when this step occurs and so in claim 13, the recitation "for at least 2 hours" is relative from an unknown source. It would be remedial to indicate at what point this step occurs.

For clarification in claim 14, the phrase --the concentration of phenylmethanesufonyl fluoride does not exceed--, is recommended as opposed to "the concentration does not exceed".

In claim 25, step b), the word "of" should be replaced by --on-- in line 5. As well in line 7, "initial volume" should also recite --of the growth medium--. In line 8 the claim should be amended to recite --agent is supplied in the growth media at a concentration of up to 0.07%--. (In claim 26 the article --an-- is necessary before antifoaming). Finally, in line 9, the claim recites that agitation is maintained, however it is not clear when the agitation occurs. It appears as if the growth phase is accompanied by agitation. However, for clarity, the time of agitation should be indicated. Similar amendment to claim 26 step b) is recommended.

In claim 26, line 5 amd 11, "the growth occurs at a pH" should recite; --the growth media during the step of growing is at a pH--.

Appropriate correction is required.

Claim Rejections - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-26, 39 and 40 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of expressing an immunotoxin in a *Pichia pastoris* that express the immunotoxin *under control of an Aox1 promoter*, the method comprising a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract,, and b) performing methanol induction on the *Pichia pastoris* in the growth medium wherein the methanol induction is performed at a temperature of 16.5°C or below, does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

The instant claims are drawn to a method of growing an immunotoxin in growth medium wherein the method requires methanol induction at a temperature below 16.5°C. The scope of

the invention is extremely broad s the immunotoxin is expressed under the control of any promoter. However, the specification teaches, "This, plus the strong AOX1 promoter employed in driving transcription of foreign genes, have made Pichia pastoris the system of choice for high levels of expression of heterologous proteins. The AOX1 promoter also has advantages in the expression of foreign proteins that are deleterious to the expressing host because the promoter is tightly regulated and highly repressed under non-methanolic growth conditions. The inducible and tightly regulated AOX1 promoter has allowed successful expression of DT based immunotoxins, in secreted form, in Pichia pastoris strains without any mutation to confer a resistance to DT. (Woo et al., 2002)." "[0083] To further compensate for Pichia pastoris protein synthesis inhibition by the expressed immunotoxin, the fermentation conditions were manipulated for full activation of alcohol oxidase I (AOX1), the rate limiting enzyme for methanol metabolism (Veenhuis et al., 1983). Since the immunotoxin gene was under the control of the same strong promoter as the AOX1 gene, the immunotoxin should be highly expressed."

Hence, the invention requires that the promoter be one that regulate expression under methanol conditions. Absent such a promoter, the method will not precede as required. The invention recites use of a broad group of promoters for regulation under methanol induction. However, the specification teaches only one such promoter that can perform this functional property. Given, the lack of adequate working examples and the lack of guidance provided by applicants, the skilled artisan would have to have conducted undue, unpredictable experimentation to practice the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 5-10, 12, 13, 15-20, 22-25, 39 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Madsen et al (US 6,723,536; see entire document) in view of Neville et al (WO 01/87982; see entire document). **This rejection is maintained for reasons of record in the office action mailed 12/12/08 and restated below.**

Applicants claim a method of expressing an immunotoxin in *Pichia pastoris* comprising growth in enzymatic digest of protein and yeast extract upon which methanol induction is performed at a temperature below 16.5°C.

Neville et al teach expression of proteins in *Pichia pastoris* wherein growth in is enzymatic digest of protein and yeast extract which methanol induction. Growth media comprises 4% glycerol, *about* 2% yeast extract, 2% enzymatic digest of protein, 1.34% yeast nitrogen base with ammonium sulfate and without amino acids, .43% PTM1 solution, wherein growth occurs at pH 3.5, and 0.01% antifoaming agent. Dissolved oxygen is about 40% (see figure 41). Methanol induction is performed at pH 7.0 wherein the agitation is 800 rpm (about 400 rpm) see page 159. Casamino acids and yeast extract serve as a source of amino acids and PMSF for at least 2 hours (it maintained expression level for 11 hours; see page 160, ¶ 2). Neville teach us of a mutant *Pichia pastoris* cell that comprises a mutation in the EF2 gene and is used for expression of A-

dmDT390-bisFV(UCHT1) (see figure 20 and page 138, ¶ 4, page 55, line 1-5). The mutation taught by Neville et al is a gly to arg at 701 (see e.g. page 54, ¶ 1).

Neville et al do not teach that the temperature is below about 16.5°C.

Madsen et al teach methods of producing recombinant proteins wherein *Pichia* cells are grown in media comprising enzymatic digestion of protein and yeast extract (see col 7-8). Methanol induction was performed wherein the induction was performed at less than about 17.5°C (see col 7, line 35-44), which range encompasses 15°C. Glycerol containing media is fed to the glycerol containing cells and dissolved oxygen is 30% (see col 8, batch glycerol phase).

As an initial point, KSR forecloses the argument that a specific teaching, suggestion or motivation is required to support a finding of obviousness. See the recent Board decision *Ex parte* Smith --USPD2d--., slip op. at 20, (BD. Pat. App. & Interfer. June 25, 2007). In the instant case, the combination of Neville et al and Madsen et al demonstrates an attempt to use known techniques to improve similar methods of protein expression using *Pichia* based upon skill that was available at the time of filing with well-established methods. In the instant case, there are multiple overlapping methods used to cultivate *Pichia* for protein expression. Madsen et al is directed to teaching methods of methanol induction in which the temperature is low. Neville et al teach methods of expressing *diphtheria toxin* using *Pichia* using methanol induction. It would have been obvious to one skilled in the art to make a substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention. Furthermore, the claims would have been obvious because a particular known technique was recognized as part of the ordinary capabilities of one skilled in the art. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and

absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 2, 4, 11, 14, 21 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Madsen et al (US 6,723,536; see entire document) in view of Neville et al (WO 01/87982; see entire document) as applied to claims 1, 3, 5-10, 12, 13, 15-20, 22-25, 39 and 40 above, and further in view of Magota et al (6,171,828; see entire document) and McGrew et al (Gene, 1997, Vol 187(2), pages 193-200; see entire document) and Chang et al (US 6,992,172; see entire document).

The teachings of Neville et al in view of Madsen et al are as above, except neither teaches specifically that methanol induction occurs by 1) limited methanol feed of 0.5-0.75 ml/min/10L or 2) a glycerol:methanol feed wherein the ratio of glycerol to methanol is 4:1. Nor do any of the previously cited references teach use of soy digest of protein.

Magota teaches methanol induction in which methanol is fed into the culture at a rate of between 1.5 ml/L/hr and 4.7 ml/L/hr (see figure 4) which correlates for a 10L culture to 0.25 to 0.78 ml/min.

McGrew et al teach that a glycerol:methanol feed can be used to successfully induce heterologous protein expression in *Pichia* cells wherein the ratio of glycerol to methanol is 4:1 (see table).

Chang et al teach that enhanced expression is accomplished by use of soytone which is a digest of soy protein (see e.g. col 59, line 29-55).

It would have been obvious to one skilled in the art to make a substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention. In the instant rejection, Magota et al, McGrew et al and Chang et al demonstrate that methods involving expression of proteins from *Pichia* are known and recognized to use methanol induction wherein either 1) limited methanol feed of 0.5-0.75 ml/min/10L or 2) a glycerol:methanol feed wherein the ratio of glycerol to methanol is 4:1. As well, Chang et al teach that soytone in the media resulted in a plant-derived (rather than animal-derived) media component that lead to increased expression of recombinant protein (see e.g. 59, line 29-55). Furthermore, the claims would have been obvious because the techniques of methanol induction by 1) limited methanol feed of 0.5-0.75 ml/min/10L or 2) a glycerol:methanol feed wherein the ratio of glycerol to methanol is 4:1 as well as use of soytone were recognized as part of the ordinary capabilities of one skilled in the art. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Argument

Applicants' arguments filed 7/23/09 have been fully considered but they are not persuasive. Applicants argue that the cited references must teach or suggest the recited specific range of methanol induction temperatures (16.5°C) to render obvious the instant invention. Applicants argue that the record is clear that Neville et al do not teach the recited temperatures. However, as regards Madsen et al, applicants argue that flow rate is turned off once 20°C is met.

"Madsen teaches methanol induction occurs for 83 hours at 26°C (column 9, line 10 (for temperature) and line 27 (for duration)). Thus, Madsen does not disclose nor teach the limitation of methanol induction at 16.5°C and below." However Madsen et al teach that methanol induction *initiates* at 26°C and harvesting at 10°C. Methanol induction is carried out during drop wise decrease of the temperature to 10°C wherein when the temperature reaches below 20°C, the methanol is cut off. This is evidenced by the teachings in column 7, "To minimize foaming, the methanol and pH loops are not shutoff until the temperature is below 20°C." Hence, methanol administration, which is required for methanol induction continues until the temperature is below 20°C. Harvesting does not commence until the temperature is at 10°C.

These teachings are, "The final phase of the fermentation is the methanol induction phase. The methanol continues to be used as a carbon source and product inducer. During this phase the methanol is fed to the fermentor at a set rate of 4.5 mL/Kg/hr for--83 hours. Harvest conditions are then set, after the conditions have been achieved the fermentation process is ready for harvest. To minimize foaming, the methanol and pH loops **are not shutoff until the temperature is below 20.degree**. C. Final angiostatin concentration is approximately 500 mg/L in the supernatant. The final WCW is approximately 300 g/L." There is no requirement to how long 'induction' occurs, so the lowering of the temperature through 16.5°C and below would provide for induction during this time of temperature change.

Applicants also argue that the when the induction temperature is the yield is more than doubled when the induction temperature is changed from 23-25°C to 15°C (see page 3, paragraph [0039] for Figure legend). However, the claims are not limited to 15°C but rather recite quite broadly that the temperature must be below 16.5°C C. In fact, the yield at 17°C is not

increased at all as demonstrated in figure 15B. Furthermore, by applicants own evidence any synergistic affect may only be at 15°C, and not above. However, such an effect would inherently be a part of the method of Madsen et al. What is required is that the determination of overlapping scope between the prior art and the recited invention. In this case, because Madsen et al teach that methanol induction occurs during the drop from 26°C to 10°C, it anticipates the teachings to which applicants invoke unexpected results. The combination reference only establish that specific methods associated with the growing and expression were also known in the art.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA B. MARVICH whose telephone number is (571)272-0774. The examiner can normally be reached on M-F (7:00-4:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, PhD can be reached on (571)-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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